SOME EXPERIMENTS ON THE DEVELOPING EAR VESICLE OF THE TADPOLE WITH RELATION TO EQUILIBRATION

By

George L. Streeter, M.D.

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SOME EXPERIMENTS ON THE DEVELOPING EAR VESICLE OF THE TADPOLE WITH RELATION TO EQUILIBRATION[1]

BY

GEORGE L. STREETER, M.D.

Associate, Wistar Institute of Anatomy

WITH TWELVE FIGURES

The eventual object of the experiments reported in the following paper was the rearing of some tadpoles which had been deprived of their auditory vesicle and acoustic ganglion, either on one side alone or on both sides; that is to say, an artificial production of a unilateral and bilateral absence of the acoustic apparatus. This was done in the expectation that it might be possible to trace the central acoustic path, in this new way, and perhaps throw further light upon its course and relations. The absence of these sense organs, however, produced such definite abnormalities in the behavior of the growing larvæ and in the development of their swimming abilities that it became at once apparent that I was dealing with valuable evidence in respect to their function and its bearing on the mechanism of equilibrium. It is, therefore, deemed advisable to restrict the following paper to the physiological features of these experiments, and reserve the study of the central nervous system of the reared specimens for a later communication.

What we already know concerning the function of the vertebrate ear is based principally on experimental sectioning or stimulation of the semicircular canals, or the nerves to their ampullæ, in adult birds and fishes.[2]

[1] Read in part before the Section of Anatomy of the British Medical Association, at the meeting held in Toronto, August 21-25, 1906.

[2] For experimental work on fishes we are for the most part indebted to Lee ('93 and '98) and Lyon ('00), both of whom carried on their experiments at the Woods Hole Laboratories. Further work on fishes has just been completed at the same place by Professor Parker, whose paper I am told is now in press and will appear in the Bulletin of the U. S. Fisheries Bureau. An abstract of part of his work was read before the American Zoölogical Society (Parker, '05). A voluminous literature exists concerning experiments on higher vertebrates, particularly the pigeon, but it need not be considered here.

The fact that it is possible to experiment on the embryo and to produce at will practically a congenital absence of this organ, besides serving as a control over the experiments on adult animals, introduces a direct advantage both as regards the ease with which the operation is performed and as regards its completeness and permanence and freedom from injury of adjoining structures, the latter point being of particular importance to those who are still in doubt as to how much is due in the experiments on adults to injuries and stimulations associated with the operation and how much is purely the result of the cessation of the stimuli which normally originate in the labyrinth. Furthermore, since the labyrinth is removed during the early formative period at a time when it may be presumed that the various organs possess their greatest adaptability, it will be seen that such embryonic interference affords a most complete test of the power of functional compensation on the part of other organs.

Behavior of Normal Tadpoles

In analyzing the behavior of operated specimens it was found necessary to make a preliminary study of control tadpoles, in order to determine the normal development of motor reflexes and their coördination and the consequent establishment of equilibrium. This was done by removing the larvæ from their gelatinous capsule shortly after fertilization and following their development in tap water. In this way it was seen that in the process of learning to swim they pass through three periods, which may be named as follows:

1. Stage of non-motility, first three days.
2. Stage of spinal reflexes, fourth to sixth days.
3. Stage of equilibrium, sixth day to maturity.

The first stage, with a favorable temperature, lasts from the time of fertilization to the third or fourth day. During this time the larvæ, aside from the movement due to cilia, lie motionless on their side on the bottom of the dish and do not respond to stimuli. The second stage begins at the time when they first respond to

mechanical stimuli by flexion of the body and tail.[1] These reactions consist of simple motor reflexes at first, but they soon become combined and coördinated so that by a series of such body flexions they are able to wiggle rapidly forward on the bottom of the dish. This manner of progression evidently consists entirely of spinal cord reflexes and is not controlled by higher centers. In order to perform it, it is necessary for the tadpoles to touch the bottom or

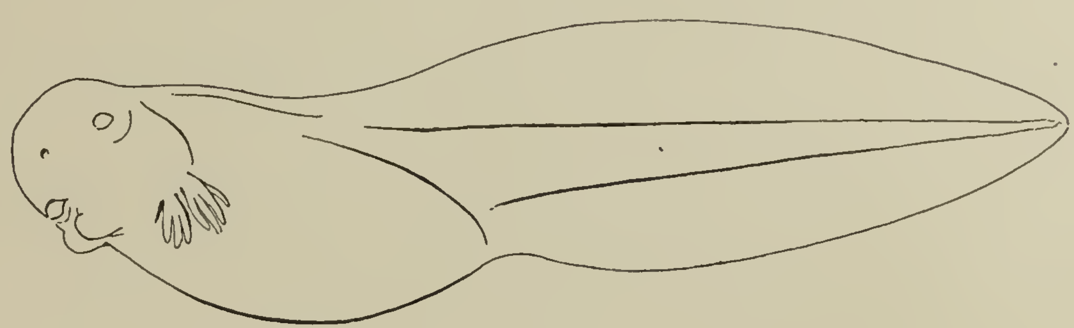

Fig. 1

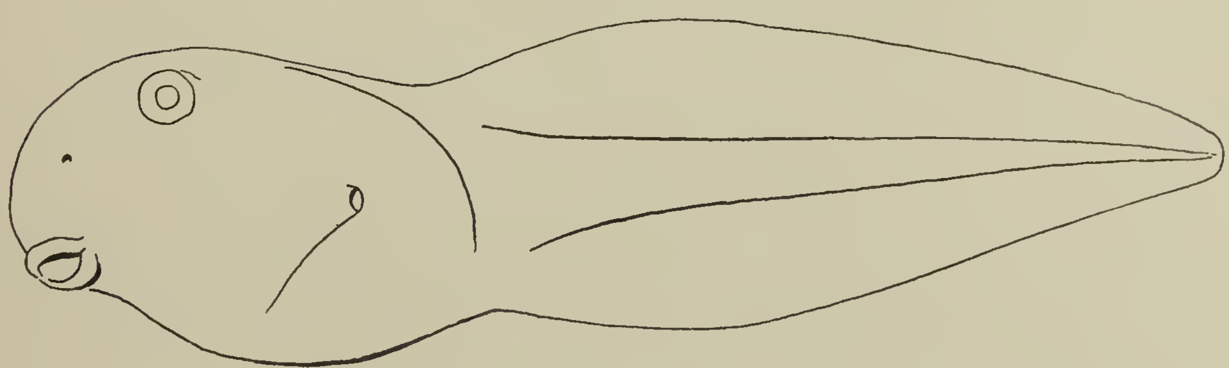

Fig. 2

Fig. 1. Outline drawing of normal tadpole (Rana sylvatica) of the second stage or stage of spinal reflexes. Enlarged 8 diameters.

Fig. 2. Outline drawing of normal tadpole (Rana sylvatica) at the beginning of the third stage. This specimen had the power of equilibration, although sections of the ear vesicle showed that the development of the semicircular canals was not yet complete. Enlarged 8 diameters.

side of the dish; when they are driven up into the free water with a pipette, where there is no contact with solid objects, they make no effort at movement, but sink inertly to the bottom; on striking the bottom they run forward again. The third stage begins when they are first able to move freely about without touching solid

[1] I have been informed by Dr. R. G. Harrison that it is just at this time that the motor nerve roots make their appearance, and this may determine the onset of the second stage. According to his observations the power of muscle contraction follows almost immediately after the development of the motor roots; but it never precedes their development, as is maintained by some. He has found the motor root present in specimens that had not yet moved.

objects. At this time a new control over their movements is developed, in virtue of which they become able to leave the bottom of the dish and swim up into free water with maintenance of what may then be called equilibrium. The form of the tadpole during the latter part of the first stage is shown in Fig. 3. The second and third stages are shown in Figs. 1 and 2.

The correlation between the histological development of the labyrinth and the development of the power of equilibrium was studied by selecting specimens of the second and the beginning of the third stages, carefully noting their behavior, and then cutting them in serial sections.[1]

From these series it could be seen that shortly before the animal enters the stage of equilibrium the labyrinth consists of a closed epithelial sac incompletely subdivided into compartments and possessing differentiated nerve endings which are connected with the brain by the acoustic nerve and ganglion. That at least one such apparatus is essential for equilibrium will be seen when I describe the behavior of tadpoles that have been completely deprived of the same. As regards the semicircular canals it is a different matter; they can already be seen in the process of development, but are not completely pocketed off until after equilibrium is already established. Consequently the semicircular canals as such are not an essential factor in equilibration.

Method of Operation

Larvæ of Rana sylvatica measuring about 3 mm. long were selected as being most suitable for the operation. Their general form at this time is shown in Fig. 3. There is a distinct tail bud, and on the head the eminences caused by the optic cup and head ganglia are visible. The structure that is to form the future labyrinth is situated just dorsal to the ganglionic eminence and is shown

[1] The correlation between the histogenesis of organs and the development of their functional activity forms a fruitful field which has been explored by comparatively few investigators. It may be approached both through ontogeny and phylogeny. Prentiss ('01) by this means worked out important facts regarding the crustacean otocyst. Many details concerning the vertebrate ear which do not belong to the scope of the present paper could doubtless be learned in the same way.

in Fig. 3 by the mark +. It consists of a cup-shaped mass of cells (auditory cup) which have differentiated themselves from the deeper layer of epidermis, and are just in the process of closing in at the edges to form the completed ear vesicle. In size this ear cup or ear vesicle is about one-half that of the optic cup.

Fig. 3. Outline drawing of Rana sylvatica at the time suitable for operation, just at the end of the non-motile stage. The tail bud is present and on the head are seen the eminences due to the optic cup and head ganglia. Above the latter is the point of operation shown by a cross. Enlarged 8 diameters.

For performing the operation it is not necessary to anesthetize the specimen as it is still in the non-motile stage and does not respond to stimulation. After removing the larva from its gelatinous capsule it is placed under a binocular microscope and an incision made near the place indicated in Fig. 3. The edge of the incision is then raised a little and the auditory cup is picked out with a needle. After a little practice one learns to make the incision directly at the edge of the cup so that it comes away easily and intact, resembling somewhat a thimbleberry. Lying just in front of it is the acoustic ganglion which is not so sharply outlined. This is also removed and, in order to make sure that it is all taken out, the surrounding mesoderm is cleaned out as far in as the brain. Where but one vesicle is to be removed the operation is then complete, and the specimen is left to proceed in its development. The wound immediately closes of itself and heals in the course of a few hours leaving no trace of the operation. Where both sides are operated on, the same procedure is carried out on both sides. The ear vesicle never regenerates following complete removal.

The ear vesicle was removed on one side from thirty specimens and on both sides from twenty specimens. The animals were then kept under observation and their behavior recorded through the whole larval period and until the completion of metamorphosis. The following notes were selected from these records.

Removal of One Ear Vesicle

Twenty-four hours after operation: Specimens are 5.5 mm. long and show presence of gill buds. In appearance and behavior, no difference can be detected between them and normal tadpoles. They lie on their side and on stimulation flex their body, but make no attempt at swimming.

Forty-eight hours after operation: Specimens are 7 mm. long, gills are branched and the blood can be seen circulating through them. In appearance and behavior they still show no departure from that seen in normal control specimens. While at rest they lie on their side. On stimulation (sunlight, jarring the dish, or touching with needle) by a rapid flexion of the body and tail from side to side they swim forward, 5–10 cm., on the bottom of the dish in a straight or slightly curved line, and then come to rest on their side, and remain so until a new stimulation excites another such excursion. Their course is directed either by the side or bottom of the dish. When forced up into free water the flexions stop and they sink inertly to the bottom.

Third day after operation: Specimens average about 8 mm. long, abdominal epidermis differentiated from that of the dorsal parts of the body by being less pigmented. Appearance and behavior is still practically normal. They begin to show a tendency to assume the upright position while at rest, but no great importance can be attached to this feature as throughout the early days of the tadpole period, preserved specimens lie in the same positions as living ones. Their posture in water may be entirely determined by their body proportions. Their movements remain of the spinal cord type seen on the previous day, the response being more prompt.

Fourth day after operation: Specimens 9–9.5 mm. long. In appearance the operated specimens are the same as the normal ones, but in behavior they present a difference. The normal ones still confine their movements to the bottom or side of the dish; when stirred up into free water, though most of them still roll about inertly, some of them are able to maintain a direct course. On the other hand the operated ones, as soon as they are driven from the bottom, swim in a spiral or circular manner as shown in the

accompanying Fig. 4. The tendency is to swim with the operated side under, and in the rolling movements around the long axis of the body it is from the operated side under to the opposite. When these same specimens touch the bottom they are able to direct their course as on the previous two days. Evidently, a functional union is normally established at about this time between the ear vesicle and the spinal cord reflex centers, upon which the individual is dependent for maintaining its position in free water, and it is not until this occurs that the removal of the ear vesicle causes any symptoms.

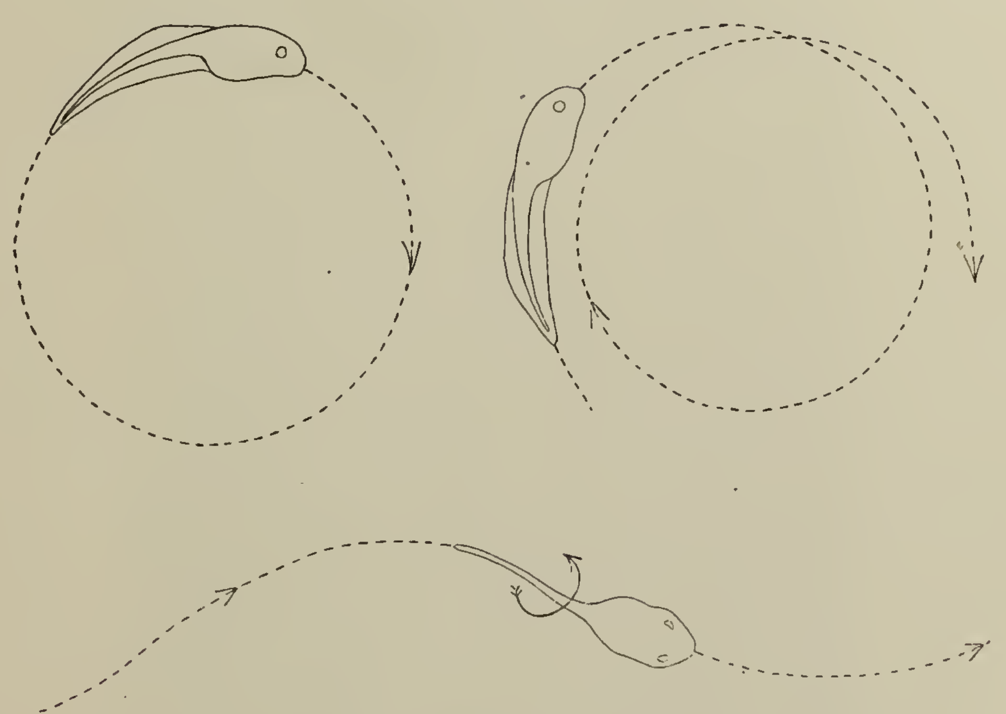

Fig. 4. Sketch showing three typical swimming movements made by specimens on the fourth day after removal of their left ear vesicle.

Sixth day after operation: Specimens about 12 mm. long, and have commenced to nibble at food and pass fæces. The characteristic movements which first appeared on the fourth day have become stronger and stand out in more marked contrast to the behavior of normal specimens which at this time can swim easily up into free water with accurate maintenance of equilibrium.

Seventh day after operation: The operated specimens show distinct improvement in swimming ability; many of them are now able to maintain a fairly direct course in free water, but on excitation they renew the spirals and circles which characterized the fourth, fifth and sixth days.

Eighth day after operation: nearly all the specimens now swim freely and directly in all parts of the water, and irregularity of swimming is only elicited by excitement.

Tenth day after operation: Swimming is practically normal. Their movements are under such control as to enable them to support themselves in free water and nibble at floating stems and leaves. It can be seen, however, that in swimming they lean

Fig. 5. Photograph of a frog whose left ear vesicle was removed when a tadpole 3 mm. long. The only asymmetry noticeable is the absence of the ear elevation on the left side normally caused by the labyrinth and its cartilaginous capsule; the lateral line on that side is straight from the eye back, while on the right or normal side it is deflected. The posture is normal. Enlarged 3½ diameters.

slightly toward the operated side, a sympton which persists throughout their larval period.

Twelfth day after operation: Specimens are normal as regards size, nourishment, and symmetry, except for the absence on the operated side of the elevation which is caused normally by the labyrinth and its cartilaginous capsule. In behavior they differ from the normal only in the slight leaning toward the operated side

and a momentary loss of equilibrium which can be elicited by excitement.

Three months after operation: The specimens passed through a normal metamorphosis at the end of the third month. A photograph made of one of them a few days after the completion of the process is shown in the accompanying Fig. 5.

As long as they continued as swimming tadpoles the slight leaning toward the operated side persisted and it was possible through excitement to cause a momentary disturbance in equilibrium, but the latter became gradually more difficult to demonstrate. As soon as they commenced to make use of their legs the character of the swimming changed; it then became a series of leg strokes instead of the sinuous flexions of the body and tail. After that it was no longer possible to detect the leaning toward the operated side; both when swimming and when at rest their behavior was to all appearance normal. When taken out of water they jumped normally and came to rest in a normal posture. When turned over on their backs they righted themselves promptly.

The fact that the slight disturbance of equilibrium, which could be still detected in the tail-swimming tadpole, could no longer be seen in the leg-swimming frog, a change completed within four or five days, probably does not signify the cure of the condition, but rather that under the latter circumstances a slight defect is more difficult to recognize. The corollary of this would be that equilibrium in the swimming tadpole is a more delicately balanced mechanism than in the kicking and jumping frog.

Removal of Both Ear Vesicles

During the first three days after the operation the appearance and behavior of these specimens are the same as seen in the normal ones, and in those from which one ear vesicle was removed. The response to stimuli is perhaps a trifle less prompt, but otherwise they could not be distinguished one from the other.

Fourth day after operation: It was seen that in one-sided operations the specimens commenced about this time to make excur-

sions into free water, and in doing it they departed from the normal by swimming in spirals and circles. Tadpoles with both ear vesicles taken out make no such excursions and show decidedly less activity. Occasionally they flex their body and tail from side to side producing a snapping effect which does not result in any forward progress. Like the other specimens they are, however, able to wiggle along in contact with the side and bottom of the dish.

Seventh day after operation: The specimens are smaller and are retarded in development as compared with the normal and one-sided specimens. They are, however, symmetrical in form and are normal as regards the appearance and movements of the eyes, mouth, heart and intestine. They are decidedly less active and stimuli produce irregular attempts at swimming, sometimes somewhat spiral in character but usually nothing more than a series of awkward flexions of the body. These flexions also occasionally occur with no apparent stimulus. They make a partially successful effort at nibbling on the bottom of the dish.

Twelfth day after operation: Absolutely no improvement in swimming; any attempt at it results in a series of somersaults. they throw their body up into the water and then promptly sink to the bottom in almost any position. When at rest, they lie on their side, back, or normally on their belly, depending apparently on whether their intestine is filled with sand, etc., to properly balance the body. The intestine is very apt to be empty because of the difficulty they experience in feeding. They do not wiggle along on the bottom as well as they did on the fourth and fifth days.

Two months after operation: The specimens could not be carried much beyond this point, the difficulty apparently being starvation from inability to wander around and collect food. Perhaps also the respiration was involved, for they were unable to go to the surface for oxygen as the normal tadpole does.

In behavior they show no improvement. For the most part they lie stiff and inert in various positions on the bottom, and their occasional attempts at swimming have never developed into anything more successful than was described on the seventh and twelfth

days after operation. Their appearance departs from the normal principally in the small contracted character of the abdominal region. In volume they are about one-third as large as the normal specimen, varying from 2.5 to 4 cm. in length. They have a hind leg bud 2.5 to 3 mm. long. As some of them commenced to die at this time the rest were put in preserving fluids for microscopial purposes.

A *summary* of the above notes on the operated individuals may perhaps be best formulated by making the following comparison with the three stages of normal behavior.

First stage: The operation was performed during the latter part, while the animals were still non-motile.

Second stage: During this period they behave exactly like normal specimens, both those having one vesicle removed and those that have been deprived of both vesicles. They respond to stimuli and learn to wiggle along in contact with the bottom of the dish in the normal manner.

Third stage: It is at the beginning of this period that they depart from the normal. It can be plainly seen from their conduct that something has happened to that controlling influence from above, which they require in order to leave the bottom and to swim and maintain their position in free water. In case but one ear vesicle is gone they swim in spirals, circles, or straight while rolling around their long axis. This, however, lasts only a few days and then it is gradually overcome. From then on they swim almost perfectly; there may be a slight tilting toward the operated side and on excitement a momentary loss of equilibrium, but this would only be seen on careful examination. It is a different matter where both labyrinths are absent; the animals in that case are completely and permanently incapacitated for swimming. There is no apparent sense of equilibrium and they never develop any. The animals were kept alive about two months, at the end of which time their movements were as irregular as at the beginning.

Transplantation of Ear Vesicle After Bilateral Removal

From the above experiments it became evident that a tadpole having but one labyrinth proceeds in its general growth and

develops swimming abilities about as well as the normal animal; but specimens deprived of both ear vesicles never learn to swim and never develop any sense of equilibrium. The next step was to see if it would be possible to remove both vesicles and at the same time transplant one of them into a new position, having in mind the successful results obtained by Lewis ('04) in transplantation of the optic cup.

After that operation if the tadpole succeeded in developing equilibrium and the power of swimming then it would prove that a transplanted ear vesicle could establish new connections with the central nervous system and develop its normal functions; the ship would simply be sailing with its compass set up in a different place.

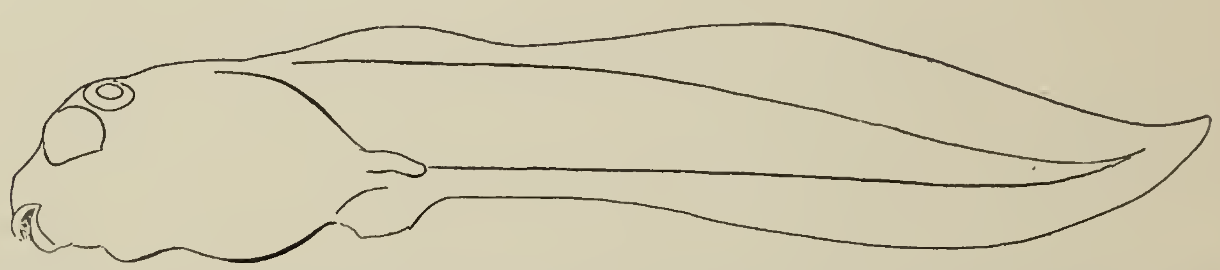

Fig. 6. Tadpole showing elevation in front of eye caused by the transplanted left ear vesicle, the right ear vesicle having been entirely removed. Drawing made three months after operation. Enlarged 4½ diameters.

The operation was one that could be performed without difficulty. A tadpole about 3 mm. long is selected and the ear vesicle taken out on one side in the manner described above. The specimen is then turned over and the opposite ear vesicle is uncovered and loosened from the epidermis. Before actually removing it a straight incision is made with scissors or needles in front of the eye and a pocket is created by gently spreading the subjacent mesoderm apart until the brain is exposed. The loosened ear vesicle is then lifted from its natural place and slipped into this pocket. If the incision is carefully made the edges of the wound close at once and on the following day there is no trace of the operation left. Nine operations of this kind were made and seven of the tadpoles successfully reared. While they were growing it could be seen from a surface view that the transplanted vesicle was developing and causing a corresponding elevation in

front of the eye. A sketch of one of these at the end of the third month is shown in Fig. 6.

Their behavior during the first week following the operation was identical with that of specimens deprived of both vesicles as was to be expected. Toward the fifth and sixth days they could make progress while touching the side or bottom of the dish, but any attempt at swimming in free water resulted only in irregular flexions of the body and somersaults. It was hoped that the transplanted vesicle might then begin to function and make it possible for them to perceive their position while in free water, but this did not occur. They continued to behave in all respects like tadpoles having no labyrinth and never gave evidence of possessing any trace of equilibrium.

At the end of the third, fourth and twelfth weeks specimens were killed in preserving fluid and prepared in serial sections. Examination of the sections showed that in six out of the seven specimens which were cut, the transplanted vesicle had developed to a greater or less extent, and it was these vesicles that formed the surface elevations that had been macroscopically visible in front of the tadpole's eyes. Graphic reconstructions of them are represented in Figs. 7 to 12. It will be seen that none of these constitute a perfect labyrinth, but on closer study it is found that they all possess certain features which are characteristic of it. In the first place, that which was transplanted in the form of an open auditory cup developed after the operation into a closed vesicle containing endolymph. This did not then remain a simple vesicle, but exhibited the tendency to subdivision into two or more compartments, the utriculus and sacculus, as seen in Figs. 7, 8, 12. In the walls of these compartments there are areas of specialized epithelium representing the maculæ acusticæ. In Fig. 7 there opens out of the more dorsal compartment a distinct endolymphatic appendage. A typical semicircular canal is not present in any of them; but what may be called a canal tendency is seen in Fig. 8, where there is a tube uniting the two principal compartments. The small blind pouches leading off the main vestibule, three of which are present in Fig. 10, doubtless represent abortive canals. In transverse section they are perfectly round and look like typical

canals. It may be recalled that Rüdinger ('88) described the semicircular canals as developing in the form of blind tubes sprouting out from the general vesicle. It is quite possible that he was dealing with an abnormal embryo and had the same form of canals that we see in Fig. 10.

The ear vesicles are more or less completely enveloped in connective tissue membranes and they are partly incorporated in masses of cartilage, some of which belongs to the normal cartilaginous cranium and some of it is the regular cartilaginous capsule of the labyrinth, the two fusing together in some places.

In four cases (Figs. 7, 8, 9 and 10) a group of ganglion cells and nerve fibers are attached to the median side of the vesicle near its caudal end and extend toward the central nervous system. In one instance (Fig. 7) the nervous connection between vesicle and brain at the junction of olfactory lobe and fore-brain, is complete, though it is only a few fibers that actually enter the brain. As the acoustic ganglion at the time of the operation is attached to the auditory cup some of its ganglion cells are undoubtedly carried along with it, and it is probable that it is these cells that furnished the nerve connections just described. At the time the transplanted ear cup was slipped into its pocket the adherent ganglion cells must have been lodged in various positions as regards the ear cup and the fact that they all come finally to lie on the median side of the vesicle and lead toward the brain must be explained by some theory of an attraction existing between brain and nerve.

When we have to deal with a transplanted labyrinth that has reached a development equal to those that function in young tadpoles, and has established communication with the central nervous system, we might expect that it would show some sign of physiological activity. The failure of it to do so is perhaps best accounted for by the fact that the point of entrance into the brain is so far away from the hind-brain centers and the spinal cord that connections with these are not established. If the experiments were varied and the vesicle transplanted to some point in the neighborhood of the occipital nerves this difficulty would be obviated.

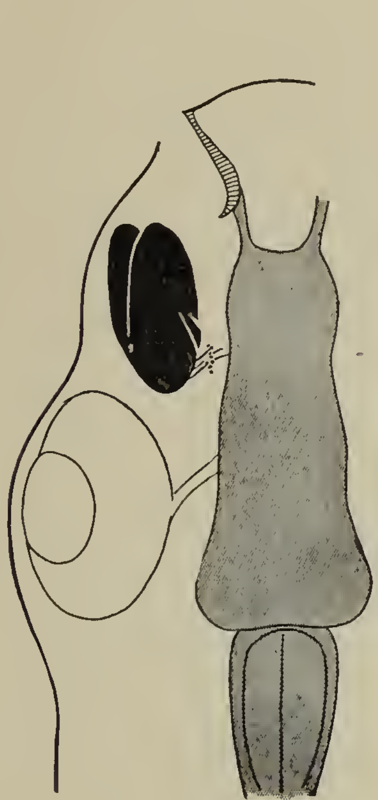

Fig. 7

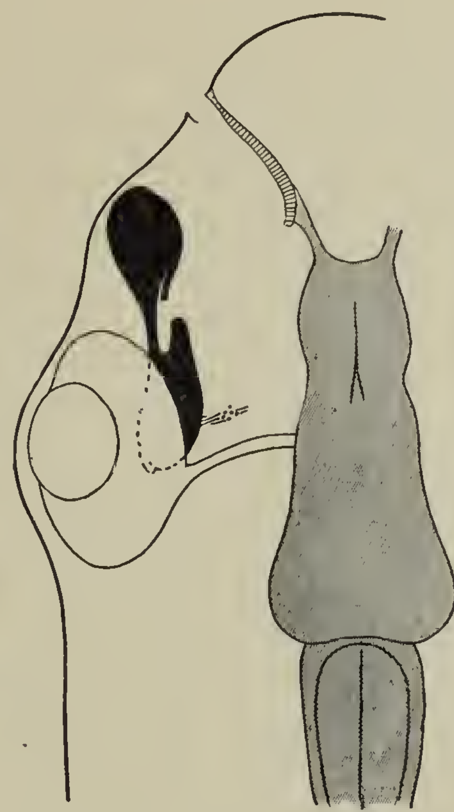

Fig. 8

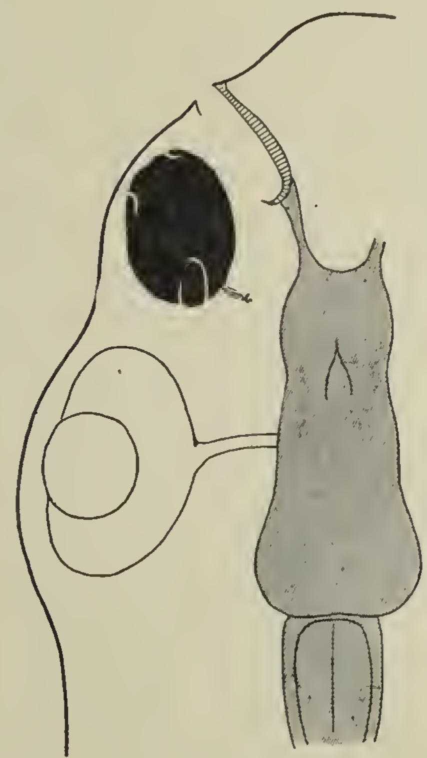

Fig. 9

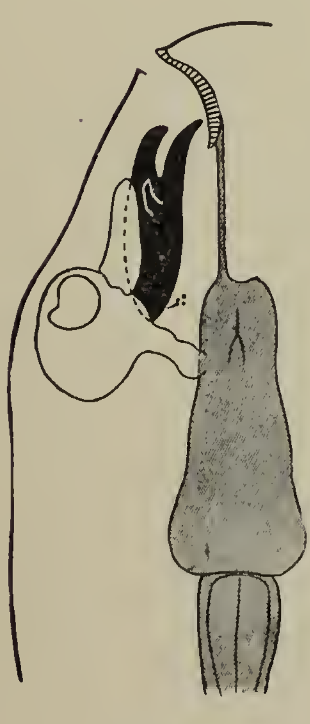

Fig. 10

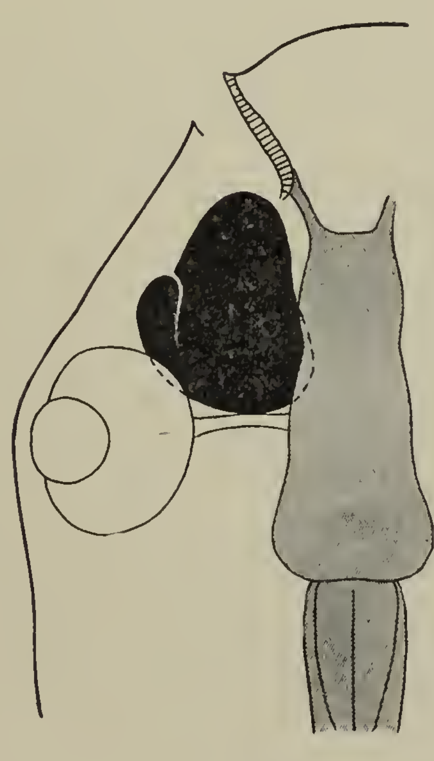

Fig. 11

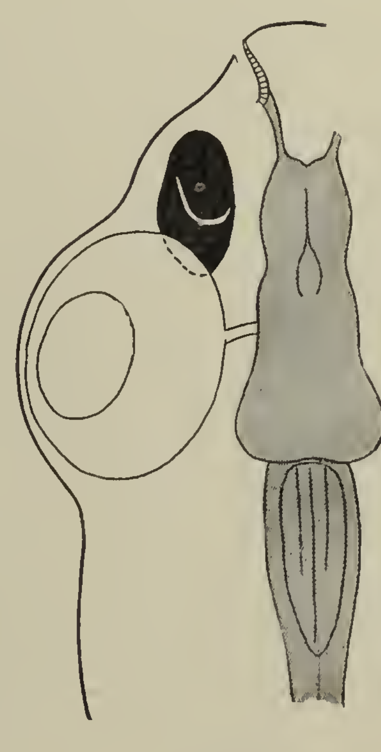

Fig. 12

Figs. 7 to 12. Graphic reconstructions showing the form and relations developed by transplanted ear vesicles, one to three months after the operation. In all six cases the right ear vesicle was removed and the left vesicle transplanted into a subdermal pocket between eye and nostril. In Figs. 7, 8, 9 and 10 the acoustic nerve and ganglion extended from ear vesicle toward brain; in Fig. 7 the connection was complete, the fibers entering at junction of fore-brain and olfactory lobe. Central nervous system, shaded; ear vesicle, solid black.

Conclusions

In the tadpole the ear vesicles are essential for the development of the power of equilibration, but the study of normal specimens shows that well developed equilibration may be present before the completion of the semicircular canals; the latter as such are therefore not essential.

When both vesicles are removed no other organ compensates for their loss and the animal is completely and permanently helpless as regards the maintenance of equilibrium. When only one ear vesicle is taken out the remaining vesicle is capable of performing the work of both so perfectly, that the casual observer would mistake them for normal individuals.

Transplantation of the ear vesicle shows that the group of cells forming the auditory cup or primitive ear vesicle is specialized to that degree that although removed from their natural relations and placed in a new environment they still continue to differentiate themselves into a structure approximating the normal labyrinth. A nerve and ganglion develops, and complete nervous connection may be established between the transplanted vesicle and the brain at an abnormal place. Where the latter occurred it did not give evidence of any functional ability.

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